In Vitro Toxicity Assessment Technique for Volatile Substances Using Flow-Through System

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Abstract: The U.S. EPA is responsible for evaluating the effects of approximately 80,000 chemicals registered for use. The challenge is that limited toxicity data exists for many of these chemicals; traditional toxicity testing methods are slow, costly, involve animal studies, and cannot keep up with the growing chemical registry. High Throughput Screening (HTS) is an *in vitro* approach of rapidly assaying a large number of chemicals for biochemical activity using robotics and automation. In recent years, HTS has been used in order to prioritize chemicals for traditional toxicity screening or to complement traditional toxicity studies. However, no method currently exists for screening volatile chemicals such as air pollutants in a HTS fashion.

We propose an *in vitro* flow-through system capable of longitudinal sampling of volatile chemicals in high-throughput fashion by measuring volatile compounds released by cells cultured with an air-liquid interface (ALI) into air directly above the cells, termed "headspace". We are currently determining whether the BEAS-2B bronchial epithelial cell line may have altered production of gaseous molecules such as volatile organic compounds (VOCs) as well as altered cell signaling molecules such as carbon monoxide (CO) and nitric oxide (NO) due to xenobiotic-induced toxicity. Previous in vitro studies have illustrated that NO, CO and certain VOCs may be altered in certain pathophysiological conditions; however, little *in vitro* research has been conducted on how production of these gaseous molecules may be altered due to xenobiotic exposure. This system can also be used to determine possible xenobiotic exposure-induced changes in CYP450 metabolism, as measured by the release of a gaseous metabolite.

To measure perturbations in formation of volatiles from cell culture, cells can be grown in an enclosed rotating glass roller bottle, at 37°C, with approximately 10% of cells covered by keratinocyte growth media at any one time. The remaining 90% of cells are cultured at an ALI, allowing for exposure to gas-phase xenobiotics of interest and simultaneously decreasing resistance to release of volatiles. An inlet introduces 5% CO_2 and purified air into the roller bottle, while an outlet tube acts as gaseous sampling port. VOCs are collected from outlet tube using a Tenax tube, which is then sent to a gas-chromatography mass spectrometry instrument for analysis. CO and NO collected from outlet tube will be collected in Tedlar® bag and analyzed by infrared and chemiluminescence, respectively. [This is an abstract of a proposed presentation and may not reflect official US EPA policy.]